

Appl. No. 09/924,857
Amdt. Dated April 1, 2004
Response to Office Action mailed on October 1, 2003

Parent Docket #P0378P3C6

Amendments to the Specification:

Please replace the title of the application on page 1, lines 11-12 with the following:

METHODS FOR THE TREATMENT OF MYOCARDIAL INFARCTION COAGULATION DISORDERS

Please replace the paragraph at beginning at page 1, line 16 with the following paragraph:
This application is a continuation application under 37 C.F.R. §1.53(b)(1) claiming priority to co-pending of application Serial No. 08/476,837 filed on 7 June 1995, now Pat. No. 6,274,142, which is a continuation of Serial No. 08/260,662 filed on 16 June 1994, now Pat. No. 5,589,173, which is a continuation of Serial No. 08/076,280 filed on 11 June 1993, now abandoned, which is a continuation of Serial No. 07/887,575 filed on 18 May 1992, now abandoned, which is a continuation in part of Serial No. 07/237,595 filed on 25 August 1988, now abandoned, which is a continuation in part of Serial No. 07/209,665 filed on 21 June 1988, now abandoned, which is a continuation in part of Serial No. 07/110,255, filed on 20 October 1987, now abandoned, which is a continuation in part of Serial No. 06/926,977 filed 4 November 1986, now abandoned, which applications are incorporated herein by reference.

Please replace the beginning on paragraph on page 20, line 12, with the following amended paragraph:

~~Triton~~ TRITONTM X-100 was from Calbiochem, San Diego, CA. All chemicals and reagents for preparative and analytical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Bio-Rad Laboratories, Richmond, CA. Factor IXa/Factor X reagent and S2222/I2581 were obtained from Helena Laboratories (Kabi Coatest kit, Helena Laboratories, Beaumont, CA, Catalogue No. 5293). YM 10 ultrafiltration membranes were from Amicon. Factor VII was purchased from Sigma Chemical. Crude phosphatidylcholine (lecithin granules from soya bean) were obtained from Sigma, St. Louis, Mo. All other chemicals were of reagent grade or better.

Appl. No. 09/924,857

Patent Docket #P0378P3C6

Amdt. Dated April 1, 2004

Response to Office Action mailed on October 1, 2003

Please replace the beginning on paragraph on page 21, line 31, with the following amended paragraph:

The RIP assay used 0.005 ml of sera from immunized and nonimmunized mice diluted with 0.495 ml of PBSAT (PBS containing 0.5% bovine serum albumin [BSA] and 0.1% ~~Triton~~ TRITONTM X-100). 50,000 cpm of ¹²⁵I-rTF was added and the mixture was incubated for 2 hr at room temperature. ¹²⁵I-rTF complexed with antibody was precipitated by incubating for 1 hr at room temperature with 0.05 ml of SPA beads. The SPA beads consisted of staphylococcal protein A bound to ~~sepharose~~ SEPHAROSETM CL-4B beads that had been pre-incubated with rabbit antimouse IgG and stored in PBS, 0.1% BSA and 0.02% NaN₃. The beads were pelleted, washed three times with PBSAT and counted in a gamma counter.

Please replace the beginning on paragraph on page 22, line 6, with the following amended paragraph:

The ELISA consisted of 0.1 ml of rTF (0.5 μ g/ml) in carbonate buffer pH 9.6 adsorbed to microtiter wells for 2 hr at 37°C. Further non-specific adsorption to the wells was blocked for 1 hr at 37°C with PBSA (PBS containing 5% BSA). The wells were washed 3 times with PBST (PBS containing 0.1% ~~Tween~~ TWEENTM 20) and the serum samples diluted in PBSAT was added and incubated 2 hrs. at 22.degree. C. The wells were washed 3 times with PBSAT. 0.1 ml of goat anti-mouse immunoglobulin conjugated to horseradish peroxidase was added to each well and incubated for 1 hr at room temperature. The wells were washed again and o-phenylenediamine dihydrochloride was added as substrate and incubated for 25 minutes at room temperature. The reaction was stopped with 2.5M H.sub.2 SO.sub.4 and the absorbance of each well was read at 492 nm.

Please replace the paragraph spanning page 22, line 20 to page 23, line 11, with the following amended paragraph:

On day 89 the spleen from mouse 29.1.B was harvested, disrupted and the spleen cells fused with X63-Ag8.653 (ATCC CRL 1580) non-secreting mouse myeloma cells using the

Appl. No. 09/924,857
Amdt Dated April 1, 2004
Response to Office Action mailed on October 1, 2003

Patent Docket #P0378P3C6

PEG fusion procedure of S. Fazakas de St. Groth et al., *J. Immun. Meth.*, 35:1-21 (1980). The fused culture was seeded into 4 plates each containing 96 microtiter wells and cultured in HAT (hypoxanthine, aminopterin and thymidine) media by conventional techniques (Mishell and Shiigi, Selected Methods in Cellular Immunology, W. H. Freeman & Co., San Francisco, pp. 357-363 [1980]). The anti-TF activity of culture supernatants was determined by ELISA and RIA. Twelve stable fusions (hybridomas) secreted anti-TF as determined by ELISA or antigen capture RIA described below. The hybridomas were expanded and cloned by limiting dilution using published procedures (Oi, V.J.T. & Herzenberg, L.A., "Immunoglobulin Producing Hybrid Cell Lines" in Selected Methods in Cellular Immunology, p. 351-372, Mishell, B. B. and Shiigi, S. M. [eds.], W. H. Freeman & Co. [1980]). Selection of clones was based on: macroscopic observation of single clones, ELISA and RIA. The antibody was isotyped using a Zymed isotyping kit according to the accompanying protocol (Zymed Corp.) Large quantities of specific monoclonal antibodies were produced by injection of cloned hybridoma cells in pristane primed mice to produce ascitic tumors. Ascites were then collected and purified over a protein-A Sepharose SEPHAROSE™ column.

Please replace the paragraph beginning on page 24, line 14 with the following amended paragraph:

The ascites was loaded onto a 10 ml column containing SPA Sepharose SEPHAROSE™ (Fermentech). The column was washed with 3M KCl. The mouse IgG was eluted with 3 to 4 column volumes of 0.1M acetic acid in 0.15M NaCl pH 2.8.

Please replace the paragraph beginning on page 24, line 19 with the following amended paragraph:

The antibody D3 was coupled to CNBr Sepharose SEPHAROSE™ according to the manufacturer's instructions at 3 mg IgG per ml of Sepharose SEPHAROSE™. (See Pharmacia Co. instruction manual). Transfected 293S cells were grown in a 1:1 mixture of Ham's F-12 (w/o glycine, hypoxanthine and thymidine) and DMEM (w/o glycine). Additions

Appl. No. 09/924,857

Patent Docket #P0378P3C6

Amdt. Dated April 1, 2004

Response to Office Action mailed on October 1, 2003

to the basal medium include: 10% dialyzed or diafiltered fetal calf serum, 50 nM methotrexate, 2.0 mM L-glutamine and 10 mM HEPES buffer.

Please replace the paragraph spanning page 24, line 28 to page 25, line 13 with the following amended paragraph:

A frozen vial of 293S (63/2S CISTF) is thawed in a tissue culture flask containing the described medium. When the culture reaches confluency it is trypsinized with trypsin-EDTA mixture and a small portion of the cell population was used to inoculate larger flasks. Cultures were monitored daily by phase microscopy to determine growth (percent confluency), morphology and general health. When rollerbottle cultures were confluent (usually within 5-7 days), the cells were trypsinized and counted. Cells were enumerated and their viabilities determined by the trypan blue exclusion technique. Typical cell numbers from a confluent 850 cm.^{sup.2} rollerbottle were between 1 to 4.times.10.^{sup.8} cells. Cells were suspended in 0.01M sodium phosphate, 0.15M NaCl. Cells were collected by centrifugation at 5000 rpm. Cells were resuspended in 50 mls TBS containing 1% ~~Triton~~ TRITON™ X per flask. Cultures were incubated one hour at room temperature and then centrifuged 8000.times.g for 20 min. Supernatant was loaded onto the D3 ~~Sepharese~~ SEPHAROSE™ column described above. The column was washed and eluted with 0.1M acetic acid, 150 mM NaCl and 05% Tween TWEEN™ 80.

Please replace the paragraph beginning on page 28, line 25 with the following amended paragraph:

Immunocytochemistry was performed according to the manufacturer's direction using the ~~Vectastain~~ VECTASTAIN® ABC alkaline phosphatase system (Vector, Inc., Burlingame, Calif.). The final reaction product was stained with the alkaline phosphatase substrate kit I to give a final stain that appeared red. The anti-tissue factor protein antibody used was antibody RD010, described in Example 2 below.

Please replace the paragraph beginning on page 29, line 1 with the following amended paragraph:

Appl. No. 09/924,857

Patent Docket #P0378P3C6

Amdt. Dated April 1, 2004

Response to Office Action mailed on October 1, 2003

An IgG fraction of the preimmune serum was used as a control for the tissue factor protein immunohistochemistry at the same IgG concentration as RD010. This was prepared by passing the preimmune serum over a protein A-Sepharose SEPHAROSE™ column. Frozen aliquots of all the antibody preparations were stored at -20°C until use.

Please replace the Abstract of the Disclosure beginning on page 45, line 6 with the following amended paragraph:

The present invention related to a method and therapeutic composition for the treatment of myocardial infarction-coagulation disorders comprising administration of a lipoprotein associated coagulation inhibitor tissue factor protein antagonist and a thrombolytic agent.